Food Chemistry 141 (2013) 2802–2811

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Buckwheat honeys: Screening of composition and properties

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ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 21 May 2013
Accepted 23 May 2013
Available online 1 June 2013

Keywords:
Buckwheat honey
Physicochemical parameters
Phenolic compounds
HPLC–DAD–MS
Volatile compounds
Solid-phase microextraction (SPME)
GC–MS

ABSTRACT

The quality of 10 buckwheat honeys, collected from Italian and est European beekeepers declaring to produce monofloral honey, were evaluated by means of their pollen, physicochemical, phenolic and volatile composition data. The results of the traditional analyses and in particular electrical conductivity, optical rotation, pH and sugar composition revealed some poorly pure samples that could not fit in the buckwheat tipology. Honey volatiles, analysed by solid phase microextraction (SPME) and gas chromatography–mass-spectrometry (GC/MS), showed more than 100 volatile compounds, most of them present in all honey samples but with quantitative variation. Besides many furfural derivates, 3-methylbutanoic acid was the main volatile compound found in most of honeys. Also the presence of 2- and 3-methylbutanal and pheynalcetaldehyde confirmed the typical buckwheat aroma of some studied samples, corroborating physicochemical data. The HPLC phenolic profile was similar across the samples and *p*-hydroxybenzoic and *p*-coumaric acids proved to be the main components.

1. Introduction

Buckwheat honey, collected from the little pink flowers by honeybees, is characterised by a dark purple colour, almost black and by a strong animal odour, malty aroma and molasses flavor (Dalby, 2000). In several studies have been reported the high antioxidant properties of this honey (Gheldof, Wang, & Engeseth, 2002), as well as its antibacterial activity, highlighting its efficacy in respiratory tract infection (cough) and wound healing (e.g. burn wounds and venous leg ulcers) (Paul et al., 2007; Ranzato, Martinotti, & Burlando 2012; van den Berg et al., 2008).

Buckwheat unifloral honey is mainly produced in North America (Canada and California), China and in some countries of Europe, such as Poland, Russia, The Netherland and Germany. Because of the quite low cultivation of buckwheat plants, in Italy the monofloral buckwheat honey is difficult to produce and it is usually found as a natural component of multifloral honeys.

The botanical and geographical origin of honey, which influence its quality and price, has been traditionally identified by the analysis of the bee pollen present in the honey, together with organoleptic and physicochemical determinations. However, this information should be enriched with data derived from the sensory profile and bioactive components. Therefore, new analytical methods have also been developed (Anklam, 1998; Ferreres, Tomás-Barberán, Gil, & Tomás-Lorente, 1991) and among them, the determination of specific markers, such as phenolic compounds, is one of the most promising way for studying the healthy properties and quality of honeys (Gómez-Caravaca, Gómez-Romero, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2006; Gómez-Caravaca, Segura-Carretero, & Fernández-Gutiérrez, 2006; Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001; Yao et al., 2003).

Besides, the honey volatile fractions have been studied and different authors report that the same volatile components are present in the majority of honeys, although the mutual proportions of these substances can be different (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2007; Anklam, 1998; Baroni et al., 2006; Piasenzotto, Gracco, & Conte, 2003; Radovic et al., 2001; Soria, Martínez-Castro, & Sanz, 2003). Similarly, certain specific volatile compounds are characteristic of a given floral origin (Cuevas-Glory, Pino, Santiago, & Sauri-Duch, 2007).

Buckwheat honey is produced at different levels of "uniflorality", following the major or minor diffusion of the corresponding plant. In Italy taking into account the great attention in healthy foods, there is a growing interest for the production of foods made from buckwheat especially for celiacs, therefore farmers are planning more extensive cultivations and consequently the output of buckwheat honey will increase. There is a lack of investigation about this interesting botanical origin so the principal aim of this study was to analyse the quality of 10 buckwheat honeys collected from different producers, prefering those that come from areas where the cultivation is wide as in east Europe. The chemical composition of samples was evaluated by the use of traditional

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http://dx.doi.org/10.1016/j.foodchem.2013.05.102

(melissopalynological and physicochemical analysis) and new analytical methods (phenolic and volatile compounds), emphasising correlations among the obtained data and also the presence of possible chemical markers of this honey.

2. Materials and methods

2.1. Honey samples

Ten honey samples were bought from beekeepers claiming to produce monofloral buckwheat honeys. Honeys were kept at room temperature (25 ± 2 °C) and in the dark until analysed.

2.2. Reagents and chemicals

Unless otherwise stated, solvents and reagents used were of analytical grade and were from Merck (Darmstad, Germany). The standards used for the identification and quantification of phenolic acids and flavonoids were: protocatechuic acid, *p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, benzoic acid, apigenin, galangin from Sigma–Aldrich (St. Louis, MO, USA), caffeic acid, *p*-coumaric acid, ferulic acid, quercetin, chrysin and pinocembrin from Fluka (Buchs, CH). The Amberlite XAD-2 was purchased from Supelco (Bellefonte, PA, USA). 5-Hydroxymethylfurfural (HMF) for HPLC analysis was purchased from Dr. Ehrenstorfer GmbH (Germany) and sugar standards for GC analysis from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Melissopalynological analysis

Melissopalynology was essentially performed according to the Italian standard UNI 11299:2008 method. The microscopic elements were concentrated by centrifuging the honey diluted in water, and the sediment examined and evaluated under the microscope. To determine the *Fagopyrum esculentum* pollen frequency, at least 300 grains were counted per sample (Von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004).

2.4. Physicochemical and sugar analyses

Physicochemical properties were analysed according to the harmonised methods of the International Honey Commission (IHC) (Bodagnov, 2009). The evaluated parameters were: water content (%), determination of specific rotation ($[\alpha]_D^{20}$), electrical conductivity (mS/cm), pH and acidity (meq/kg), hydroxymethylfurfural (HMF) content (mg/kg), diastase activity (Gothe degrees) and colour (mm Pfund).

Water content was ascertained with an Abbe refractometer reading at 20 °C, obtaining the corresponding value of moisture from the Chatway table. Specific rotation was measured using a digital polarimeter (Euromex, Holland). Electrical conductivity was determined with a CON 700 OAKTON® conducimeter (Vernon Hills, USA), whereas pH and acidities (free, lactonic and total) with an Automatic Potentiometric Titrator AT-610 (KEM, Japan). The assessment of honey freshness involved the determination of hydroxymethylfurfural (HMF) and diastase activity. HMF quantification was performed using a Jasco HPLC (mod.PU-2080; Tokyo, Japan) equipped with UV detector (285 nm) and a reverse phase column (Synergi 4 μm Max-RP, 150 × 4.6 mm; Phenomenex). The diastase activity was measured using the Phadebas® Honey Diastase Test (Magle Life Sciences International, Lund, Sweden). Finally colour was determined using a Honey Colour Analyser (mod.C221; Hanna Instruments, Padova, Italy) and the results were expressed using the Pfund scale.

Sugars were determined using the GC method reported by IHC, followed by the I.N.A derivatisation procedure. The analysis was performed with a Fisons Carlo Erba Instruments GC 8000 (Rodano, MI, Italy) gas chromatograph equipped with a SE 52 capillary column (MEGA Legnano, MI, Italy), an "on column" injector and a flame ionisation detector. The oven temperature was held at 70 °C and programmed at 50 °C/min linear ramp to 100 °C, 7 °C/min to 330 °C, 50 °C/min to 350 °C and held 5 min. The following sugars were analysed: fructose, glucose as monosaccharides; sucrose, maltose, isomaltose, trehalose and gentiobiose as disaccharides; erlose, panose, maltotriose, palatinose, isomaltotriose, raffinose, melezitose and maltotetrose as polysaccharides. Their identification and quantification in honey samples were obtained by comparison with a mix of standards eluted under the same conditions and they were expressed as g/100 g.

2.5. Phenolic compounds

2.5.1. Sample extraction

Phenolic compounds for HPLC analysis were extracted from honey as described previously (Martos, Cossentini, Ferreres, & Tomás-Barberán, 1997) with some modifications reported by Gómez-Caravaca, Gómez-Romero, et al. (2006), Gómez-Caravaca, Segura-Carretero et al. (2006). About 30 g of honey was dissolved with five parts (150 ml) of acidified water (pH 2 with HCl), stirring at room temperature until completely fluid. The solution was mixed with 40 g Amberlite XAD-2 (pore size 9 nm, particle size 0.3–1.2 mm) and stirred for 10 min, which was considered enough to adsorb honey phenolics with a recovery rate more than 80% (Martos et al., 1997). The Amberlite particles were then packed in a glass column (30 cm × 3 cm), washed with acidified water (pH 2 with HCl, 100 ml) and subsequently rinsed with distilled water (300 ml). The phenolic compounds remained adsorbed on the column (Ferreres et al., 1991), while sugars and other polar compounds eluted with the aqueous solvent. The whole phenolic fraction was then eluted with methanol (300 ml) and taken to dryness under reduced pressure (50 °C). The residue was re-suspended in distilled water (5 ml) and extracted with diethyl ether (5 ml × 3). The ether extracts were combined, concentrated under reduced pressure at 30 °C, and dissolved in 1 ml of 50:50 (v/v) methanol:water solution. Finally all extracts were filtered through a 0.45 μm mesh and analysed by HPLC–MS.

2.5.2. HPLC–DAD–MS analysis

All HPLC analyses were performed using an HP 1100 Series Instrument (Hewlett–Packard, Wilmington, DE) equipped with a binary pump (model G1312A) delivery system, a degasser (model G1322A), an autosampler (Automatic Liquid Sampler, ALS, model G1313A), a HP diode-array UV–VIS detector (DAD, model G1315A) and a HP-single-quadrupole mass spectrometer detector (MS, model G1946A). Separations were carried out on a reverse phase column Eclipse XDB-C_{18}, 5 μm 250 × 3.0 mm ID, (Agilent Technologies, Santa Clara, CA, USA) with a Securityguard precolumn filter. All solvents were HPLC-grade and filtered with a 0.45 μm filter disc. Elution was carried out with a 1% acetic acid (solvent A) and acetonitrile (solvent B) and with a linear gradient starting with 5% B, to reach 7% at 5 min 9% at 10 min, 12% at 15 min, 15% at 18 min, 16% at 20 min, 18% at 25 min, 20% at 30 min, 22% at 32 min, 25% at 35 min, 28% at 38 min, 30% at 40 min, 31% at 42 min, 32% at 45 min, 34% at 48 min, 35% at 50 min, 40% at 55 min, 50% at 60 min, 95% at 70 min and 5% at 75 min, and post run for 5 min. All the analyses were carried out at room temperature, with an injected volume of 10 μl and a flow rate of 0.5 ml/min. UV spectra were recorded from 210 to 600 nm, whereas the chromatograms were monitored at 280 and 330 nm, since the majority of the honey flavonoids and phenolic acids show

their UV absorption maxima around these two wavelengths (Martos et al., 1997).

MS analyses, performed with the same HPLC–DAD condition, were carried out using an electrospray ionisation (ESI) interface in positive and negative mode at the following conditions: drying gas flow (N_2), 11.0 L/min; nebulizer pressure, 60 psi; gas drying temperature, 350 °C; capillary voltage, 4000 V; fragmentor voltage, 90 V; scan range, *m/z* 120–850. Ionisation of the phenolic compounds in negative mode gave high sensitivity and selectivity and ionisation in positive mode provided extra certainty in the determination of the molecular masses.

The honey phenolic acids and flavonoids were identified by chromatographic comparison with reference standards and by matching their UV spectra with those of the markers. The individual phenolic compounds were quantified by their absorbance in the HPLC chromatograms against external standards, using ferulic acid at 280 nm for phenolic acids and chrysin at 330 and 280 nm for flavonoids.

The limit of detection (LOD) and the limit of quantification (LOQ) were 0.06 and 0.21 μg/ml, respectively, for phenolic acids and 0.07 and 0.23 μg/ml for flavonoids, as reported in Table 1.

Integration and data elaboration were performed using HP ChemStation software (Hewlett–Packard, Palo Alto, CA). The quantitative analyses were carried out in triplicate and expressed as micrograms per 100 g of honey.

2.6. Volatile compounds

2.6.1. Isolation of volatile compounds

The isolation of the aroma compounds was performed using a solid phase microextraction (SPME) procedure. A manual SPME holder (Supelco, Bellefonte, PA, USA) with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm, coating 1 cm) fibre (Supelco) was used to extract headspace volatiles from honey. Prior to use, the fibre was conditioned at the GC injection port, at a temperature of 250 °C for 1 h.

For each extraction 1.5 g of honey were introduced into a 10 ml vial and diluted 1:1 with distilled water (30% NaCl, w/v). The vial was hermetically sealed with a PTFE/silicone septa and sonicated for 5 min. After homogenisation, the vial was maintained in a water bath at 60 °C during equilibration (15 min) and extraction (40 min) and was partially submerged so that the liquid phase of the sample was in the water. All the experiments were performed under constant stirring velocity by magnetic stirrer.

After sampling, the SPME fibre was removed from the vial and inserted into the GC–MS injector (250 °C) for 5 min where the extracted volatiles were thermally desorbed directly to the GC column.

2.6.2. GC–MS analysis

The GC–MS analyses were performed on a GCMS-QP2010 Plus (Shimadzu Corp., Tokyo, Japan) equipped with a 30 m × 0.25 mm i.d., with a 0.25 μm film thickness, RTX-WAX capillary column (Restek, Bellefonte, PA). Samples were injected with a split ratio of 1:10 and helium at 1 ml/min was used as carrier gas. Oven temperature was held at 40 °C for 10 min and raised to 200 °C at 3 °C/min for 3 min and then to 250 °C at 10 °C/min, for 2 min. Mass spectra were recorded in electron impact (EI) ionisation mode at 70 eV, scanning the 33–500 *m/z* range. Interface and source temperature were 210 and 230 °C, respectively.

Identifications were based on comparison of the obtained spectra with those of the libraries NIST05 Mass Spectral Library and with published data, and were confirmed, when possible, by using Kovats retention indices (RI). Retention indices were calculated for each compound using homologous series of C9–C19 n-alkanes. The relative amounts of individual components were directly obtained from total ion current (TIC) peak area and expressed as percent peak areas (RPA %). The component percentages were calculated as mean values from duplicate GC–MS analyses.

2.7. Statistical analysis

Relative standard deviation was obtained, where appropriate, for all data collected. One-way analysis of variance, ANOVA (Tukey's honest significant difference multiple comparison) was evaluated using Statistica 8 software (2006, StatSoft, Tulsa, OK, USA). *p* values lower than 0.05 were considered statistically significant. All chemical analyses were carried out in triplicate, and the analytical data were used for statistical comparisons.

3. Results and discussion

3.1. Physicochemical and melissopalynological analysis

Table 2 lists the mean, standard deviations and ranges of the data obtained from the analysis of the different physicochemical parameters.

The electrical conductivity of honey usually shows great variability according to the floral origin and the results obtained for the samples under study varied between 0.33 and 1.86 mS/cm. In particular, the values of samples B-1, B-9 and B-10 (1.02, 1.37 and 1.86 mS/cm, respectively) exceeded the limit allowed for floral honeys (0.8 mS/cm), suggesting the likely presence of honeydew (Bentabol Manzanares, Hernández García, Rodríguez Galdón, Rodríguez Rodríguez, & Díaz Romero, 2011; Vela, Lorenzo, & Pérez, 2007).

Moisture content remarks the fact that sample B-1, B-9 and B-10 are supposed to be predominantly honeydew, indeed they showed the lowest water values, around 16%; however none of the honeys exceeded the permitted limit established by the European Community Directive (The Council of the European Union, 2002).

Honey has the property of rotating the polarisation plane of polarised light. Floral honeys are levorotatory in contrast to honeydew and adulterated honeys, which are usually dextrorotatory. This is a consequence of the normal preponderance of fructose in floral honey, which shows a negative specific rotation over glucose. Only the three samples B-1, B-9 and B-10 were dextrorotatory, while other samples were levorotatory.

Most of the studied honeys were acidic, having a pH in the range 3.8–4.9. Usually pH values range between 3.5 and 4.5 in nectar honey and between 4.5 and 5.5 in honeydew. Suspected honeydew samples (B-1, B-9 and B.10) showed the highest values (4.8, 4.8 and 4.9), in agreement with the literature (Bentabol Manzanares et al., 2011; Vela et al., 2007).

Table 1
Analytical parameters of the HPLC proposed method.

Standard compound	RSD (%)	LOD (μg/ml)	LOQ (μg/ml)	Calibration range (μg/ml)	Calibration equation	r^2
Ferulic acid (280 nm)	0.40	0.06	0.21	1.5–250	$y = 59.622x - 67.579$	0.9997
Chrysin (330 nm)	0.40	0.07	0.23	2.5–250	$y = 54.737x - 76.34$	0.9996

Table 2
Pollen analysis, physicochemical parameters and sugar composition in the buckwheat honey analysed.

Parameter	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10	Mean	Range	SD[a]
Pollen analysis (% *Fagopyrum esculentum*)	0.3	7.3	5.0	15.3	6.0	18.3	11.0	42.0	47.0	44.7	–	–	–
Moisture (%)	16.0	18.5	18.0	17.4	17.1	17.4	17.5	17.4	16.6	16.4	17.2	16.0–18.5	0.74
Electrical conductivity (mS/cm)	1.02	0.37	0.36	0.45	0.38	0.33	0.42	0.35	1.35	1.86	0.69	0.33–1.86	0.54
Colour (mm Pfund)	107	127	150	150	147	150	150	150	150	150	143	107–150	15
HMF (mg/kg)	1.7	0.9	5.4	22.3	2.2	2.5	6.4	262.6	2.4	0.6	30.7	0.6–262.6	81.7
Free acidity (meq/kg)	22.9	41.4	19.2	30.0	22.5	25.7	32.1	24.3	31.3	50.3	30.0	19.2–50.3	9.6
Lactonic acidity (meq/kg)	2.0	8.5	5.4	9.1	7.4	7.8	11.0	7.4	8.4	6.5	7.4	2.0–11.0	2.4
Total acidity (meq/kg)	24.9	49.9	24.6	39.1	27.9	33.5	43.1	31.7	39.7	56.9	37.1	24.6–56.9	10.7
pH	4.8	3.8	4.0	4.0	4.2	4.1	3.9	3.8	4.8	4.9	4.2	3.8–4.9	0.4
Diastase (°Gothe)	24.8	35.5	20.3	22.0	32.6	20.2	35.2	22.5	42.4	52.3	30.8	20.2–52.3	10.8
Specific rotation $[\alpha]_D^{20}$	1.8	−14.3	−15.6	−9.5	−8.5	−11.5	−11.6	−15.0	1.1	10.25	−7.3	−15.6–(10.3)	8.7
Fructose (g/100 g)	36.0	38.4	38.1	37.5	39.0	37.1	37.4	39.4	35.9	30.6	36.9	30.6–39.4	2.5
Glucose (g/100 g)	29.4	35.1	34.3	33.2	32.4	31.9	33.8	28.9	31.3	27.3	31.7	27.3–35.1	2.5
Sucrose (g/100 g)	0.4	0.1	0.9	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1–0.9	0.3
Maltose (g/100 g)	4.3	2.4	3.0	5.0	5.0	5.5	4.2	6.5	6.7	6.8	4.9	2.4–6.8	1.5
Trehalose (g/100 g)	1.5	n.d.	n.d.	0.1	0.1	n.d.	0.1	n.d.	0.1	0.1	0.2	0.0–1.5	0.5
Isolmaltose (g/100 g)	1.0	0.4	0.8	1.4	1.0	0.7	0.5	1.0	1.5	2.1	1.0	0.4–2.1	0.5
Gentiobiose (g/100 g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.3	0.1	0.0–0.3	0.1
Melezitose (g/100 g)	1.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.1	0.2	0.0–1.6	0.5
Raffinose (g/100 g)	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.0–1.1	0.4
Erlose (g/100 g)	1.7	0.1	0.1	0.2	0.8	n.d.	0.1	n.d.	0.6	0.8	0.4	0.0–1.7	0.5
Maltotriose (g/100 g)	0.1	n.d.	n.d.	0.1	0.1	n.d.	n.d.	n.d.	0.4	1.3	0.2	0.0–1.3	0.4
Panose (g/100 g)	0.1	n.d.	0.1	0.1	0.2	n.d.	n.d.	0.1	0.4	0.6	0.2	0.0–0.6	0.2
Isomaltotriose (g/100 g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0–0.0	0.0
Maltotetrose (g/100 g)	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	0.1	0.0–0.5	0.2
Total sugar (g/100 g)	77.3	76.5	77.3	77.7	79.0	75.3	76.2	76.0	77.8	70.0	76.2	70.1–79.0	2.4
Fructose/glucose ratio	1.2	1.1	1.1	1.1	1.2	1.2	1.1	1.4	1.2	1.1	1.2	1.1–1.4	0.1

n.d., not detected, under the limit of detection (0.1 g/100 g).
[a] Standard deviation

HMF and diastase activity are widely recognised as quality factors, evaluating ageing and temperature abuse. As reported in Table 2, all honeys fell within the limits permitted by the European Community regulations (The Council of the European Union, 2002) for diastase number and HMF content, except for sample B-8 which presented an inappropriate HMF value (262.6 mg/kg), suggesting inadequate storage or processing.

In the tested honey samples colour values were very similar among them and ranged between 107 and 150 mm Pfund. These high values were due to the typical dark purple colour of buckwheat honey that looks almost black.

Regarding the sugars, 14 carbohydrates were identified (Table 2). The monosaccharides glucose and fructose were present in every sample and were the main sugars in all of them. Fructose was always the most important sugar quantitatively (range 31–39%), followed by glucose (range 27–35%). The suspected honeydew samples (B-1, B-9 and B-10) showed the lowest percentage of monosaccharides, in agreement with other studies (Mateo & Bosch-Reig, 1997; Terrab, Vega-Pérez, Díez, & Heredia, 2001). In relation to the disaccharides, maltose was the most abundant one, ranging from 2.4% to 6.8%, with the suspected honeydew samples B-9 and B-10 showing the higher content, as reported by Mateo & Bosch-Reig(1997). Sucrose, important sugar from a legislative point of view, had low values suggesting an advanced stage of ripening of the honeys, which would encourage the conversion of sucrose into glucose and fructose. Trehalose, isomaltose, gentiobiose were present only in some samples ranging from 0.1% to 1.5%, 0.4% to 2.1% and 0.2% to 0.3% respectively. The other polysaccharides were not present in all samples and in particular melezitose were identified only in the three suspected honey blends, as already reported in literature (Terrab et al., 2001).

As regards pollen profile, buckwheat honey is still not perfectly characterised. Microscopic analysis of samples confirmed that *F. esculentum* pollen is under-represented in honey and exhibited a wide variability and low frequences (from 0.3% to 47%). Furthermore pollens of Cruciferae family were present as secondary nectar source especially in samples from eastern Europe. Sample B-1 presented a 0.3% of *F. esculentum* pollen, so it is possible to suppose that it was not a buckwheat honey. On the other hand, B-8, B-9 and B-10 honeys showed the highest frequency, exceeding 40%. Because of late flowering of buckwheat plant, in these cases we can assume that, the *Fagopyrum* high percentage could be due to the fact that the bees collected pollen instead of *Fagopyrum*'s nectar while were doing the honeydew harvest.

3.2. Determination of phenolic compounds in buckwheat honey

Fig. 1 shows the typical HPLC chromatograms obtained at 280 and 330 nm for all samples and, as reported in Table 3, the compounds were identified on the basis of their UV and mass spectra, as well as their chromatographic behaviour compared to external standards, when available. All the tested honey samples had similar but quantitatively different phenolic profiles and up to 20 peaks could be assigned to phenolic compounds and identified as phenolic acids (peaks 1–10), abscisic acid (peaks 11 and 12) and flavonoids (peaks 13–20). Twelve compounds were present in all samples: *p*-hydroxybenzoic acid (*p*-HBA), *p*-coumaric acid, benzoic acid, abscisic acid isomers (ABA1 and ABA2) and most of the identified flavonoids, thus quercetin, apigenin, pinobanksin, kaempferol, chrysin, pinocembrin and galangin. All the identified phenolic compounds were already reported in honey (Alvarez-Suarez, González-Paramáz, Santos-Buelga, & Battino, 2010; Estevinho, Pereira, & Moreira, 2008; Tomás-Barberán et al., 2001; Truchado, Ferreres, Bortolotti, Sabatini, & Tomás-Barberán, 2008) and some of them specifically found in buckwheat honey (Biesaga & Pyrzynska, 2009; Gheldof et al., 2002; Jasicka-Misiak, Poliwoda, Dereń, & Kafarski, 2012; Ramanauskiene, Stelmakiene, Briedis, Ivanauskas, & Jakštas, 2012; Zhou et al., 2012). There were other compounds present in the samples that had similar phenolic spectra and chromatographic behaviour but they could not be identified

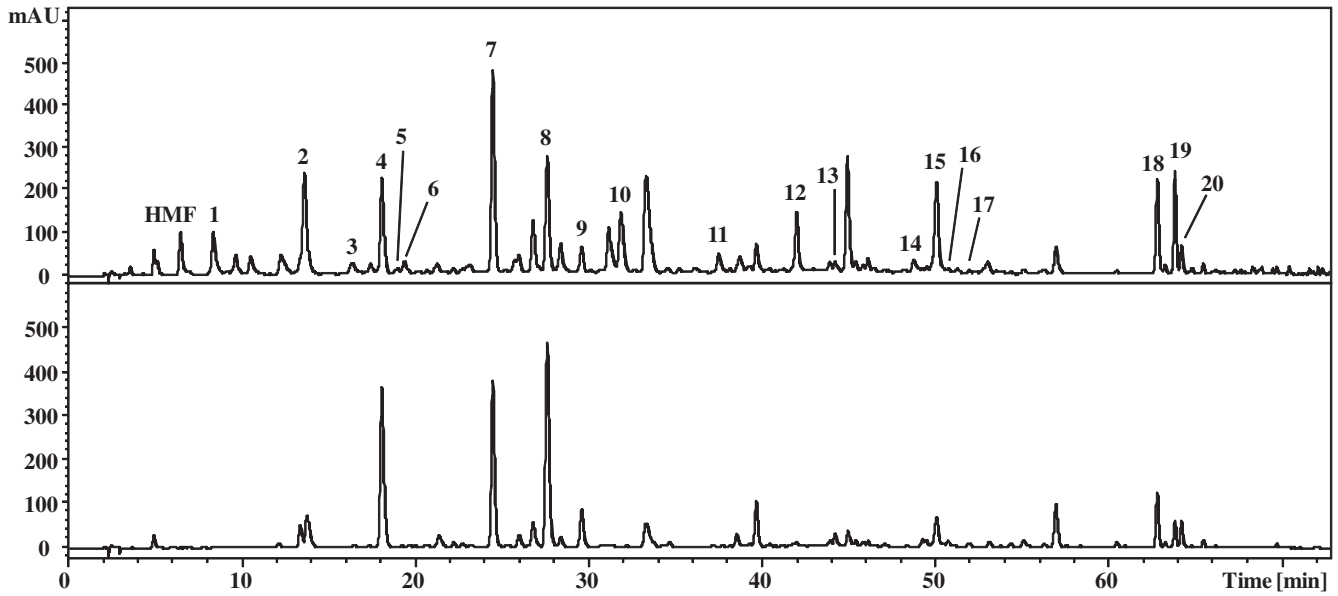

Fig. 1. HPLC–UV chromatograms at 280 nm (A) and 330 nm (B) of the identified phenolic compounds and abscisic acids in buckwheat honey extract. Peaks: HMF: 5-hydroxymethylfurfural; (1) protocatechuic acid; (2) *p*-hydroxybenzoic acid; (3) *p*-hydroxyfenilacetic acid; (4) caffeic acid; (5) unidentified compund; (6) syringic acid; (7) *p*-coumaric acid; (8) ferulic acid; (9) isoferulic acid; (10) benzoic acid; (11) *trans–trans* abscisic acid; (12) *cis–trans* abscisic acid; (13) quercetin; (14) apigenin; (15) pinobanksin; (16) kaempferol; (17) isorhamnetin; (18) chrysin; (19) pinocembrin; (20) galangin.

Table 3
R_t (min), UV and MS spectra from the different phenolic compounds identified in the buckwheat honeys analysed.

Peak	Phenolic compund	R_t	UV (nm)	Mass data ESI$^-$	Mass data ESI$^+$
1	Protocatechuic acid	8.4	230, 260, 296	153 $[M-H]^-$	155 $[M+H]^+$, 193 $[M+K]^+$
2	*p*-Hydroxybenzoic acid	13.6	254	137 $[M-H]^-$	139 $[M+H]^+$, 161 $[M+Na]^+$, 177 $[M+K]^+$
3	*p*-Hydroxyfenilacetic acid	16.3	228, 276	151 $[M-H]^-$	153 $[M+H]^+$, 191 $[M+K]^+$
4	Caffeic acid	18.0	238, 296sh, 324	179 $[M-H]^-$, 135	181 $[M+H]^+$, 203 $[M+Na]^+$, 219 $[M+K]^+$
5	Unidentified compund	19.0	226, 284	121 $[M-H]^-$	123 $[M+H]^+$
6	Syringic acid	19.4	274	197 $[M-H]^-$, 153	199 $[M+H]^+$
7	*p*-Coumaric acid	24.4	232, 310	163 $[M-H]^-$	165 $[M+H]^+$, 187 $[M+Na]^+$, 203 $[M+K]^+$
8	Ferulic acid	27.6	240, 296, 322	193 $[M-H]^-$, 134, 149, 178	195 $[M+H]^+$, 217 $[M+Na]^+$, 233 $[M+K]^+$
9	Isoferulic acid	29.5	234, 294, 322	193 $[M-H]^-$, 134, 149	233 $[M+K]^+$
10	Benzoic acid	31.8	234, 274	121 $[M-H]^-$	123 $[M+H]^+$, 161 $[M+K]^+$
11	*trans–trans* Abscisic acid	37.5	266	263 $[M-H]^-$, 153	265 $[M+H]^+$, 287 $[M+Na]^+$, 303 $[M+K]^+$
12	*cis–trans* Abscisic acid	41.9	266	263 $[M-H]^-$, 153	265 $[M+H]^+$, 287 $[M+Na]^+$, 303 $[M+K]^+$
13	Quercetin	44.2	256, 270sh, 310sh, 370	301 $[M-H]^-$, 151, 179	325 $[M+Na]^+$, 341 $[M+K]^+$
14	Apigenin	48.8	230, 268, 292sh, 338	269 $[M-H]^-$, 151, 139	271$[M+H]^+$, 309 $[M+K]^+$
15	Pinobanksin	50.0	292	271 $[M-H]^-$, 253	273$[M+H]^+$, 293 $[M+Na]^+$, 311 $[M+K]^+$, 153
16	Kaempferol	50.7	266, 292sh, 322sh, 367	285 $[M-H]^-$, 151	287 $[M+H]^+$, 325 [M+K]
17	Isorhamnetin	51.9	254, 371	315 $[M-H]^-$, 300, 151	317$[M+H]^+$, 355 $[M+K]^+$
18	Chrysin	62.7	270, 320	253 $[M-H]^-$, 151	255 $[M+H]^+$, 277 $[M+Na]^+$, 293 $[M+K]^+$
19	Pinocembrin	63.7	290, 330	255 $[M-H]^-$	257 $[M+H]^+$, 279 $[M+Na]^+$, 295 $[M+K]^+$
20	Galangin	64.1	222, 266, 316sh, 358	269 $[M-H]^-$, 151	271 $[M+H]^+$, 309 $[M+K]^+$

due to the lack of availability of standard compounds. One of them is a relevant peak in most of the chromatograms (peak 5), with a value in the range of 2–50% of the total phenolic content in all of the samples investigated. As the UV spectrum and chromatographic behaviour is quite similar to that of an hydroxybenzoic acid (Table 3), this compound was quantified at 280 nm in order to provide an approximate measure of its presence in the studied honeys.

The identity of the most of phenolic compounds could be confirmed positively by comparison with standards, whereas the peaks 9, 11, 12, 15, 16 and 17 were tentatively identified, as follows.

The compound at retention time 28.9 min reported a molecular ion at *m/z* 193 $[M-H]^-$ and two fragments at *m/z* 134 and 149, as reported by ferulic acid (peak 8) that was already identified by co-elution with its relative standard. The same data and UV behaviour was presented by Gardana, Scaglianti, Pietta, and Simonetti (2007), so this compound was identified as isoferulic acid.

Peaks 11 and 12 at 38.2 and 41.6 min, respectively, showed a molecular ion at 263 *m/z* ($[M-H]^-$) and a fragment at 153 *m/z*; Izumi, Okazawa, Bamba, Kobayashi, and Fukusaki (2009) assigned this pattern at the abscisic acid. In particular, the first peak has been assigned to the *trans–trans* abscisic acid isomer (ABA1) and the second one to the *cis–trans* abscisic acid isomer (ABA2), as already reported in different kind of honey by other authors (Ferreres, Andrade, & Tomás-Barberán, 1996; Tomás-Barberán et al., 2001; Truchado et al., 2008; Yao et al., 2003).

The mass analysis in negative mode of compound at 49.5 min showed a molecular ion at *m/z* 271 $[M-H]^-$, confirmed by the positive ionisation data with a molecular ion at 273 $[M+H]^+$ and its

adducts at *m/z* 293 $[M+Na]^+$ and *m/z* 311 $[M+K]^+$. Moreover, the negative mass spectrum reported an ion fragment with *m/z* 253 corresponding to $[M-H_2O]^-$. The loss of water is due to the presence of a hydroxyl group at C_3 of the C ring that is characteristic of flavanols. These data and the UV spectra assigned this pattern (peak 15) at the pinobanksin, as already shown by Gardana et al. (2007).

The compound at retention time 50.1 min and molecular ion $[M-H]^-$ at *m/z* 285 (peak 15) was identified as kaempferol. The identity was confirmed by its UV spectra, according to literature (Biesaga & Pyzynska, 2009; Gardana et al., 2007).

In accordance with Gardana et al. (2007) and Alvarez-Suarez et al. (2010), the registered UV and mass spectra identified the next peak at 51.2 min (peak 17) as the flavonol isorhamnetin.

3.2.1. Phenolic acids and abscisic acid content

In the 10 honey samples, the content of total phenolic acids averaged 2214.2 μg/100 g of honey. According to other authors (Biesaga & Pyzynska, 2009; Gheldof et al., 2002; Jasicka-Misiak et al., 2012; Zhou et al., 2012), *p*-coumaric acid (29.5%) and *p*-hydroxybenzoic acid (21%) were the main components with an average of 666.7 and 460.0 μg/100 g, respectively (Table 4). In addition, the unknown phenolic acid (peak 5) was present in high levels in all honeys with an average of 420.8 μg/100 g, representing up to the 20% of the total phenolic acids in buckwheat honey. The other phenolic acids were present in relatively lower amounts, ranging from 2.5% (protocatechuic acid) to 10.1% (benzoic acid) of their total.

In addition, abscisic acid has been found in all honey samples in quite large amount. The mean level of *trans–trans*-abscisic acid was 158.2 μg/100 g, whereas the level of its isomer, *cis–trans*-abscisic acid was 220.4 μg/100 g. Abscisic acid is a plant hormone which is related to the protection of plants in drought and environmental stress. This compound is known to be present in floral nectars and can therefore be present in honey in different amount, so it could be used as a biochemical marker for aiding in the authentication of floral origin of honey. Both isomers of abscisic acid have been found in various honeys, such as heather honey (400–1800 μg/100 g), rapeseed honey (25–250 μg/100 g), lime tree honey (50–500 μg/100 g) and acacia honey (100–250 μg/100 g) (Tomás-Barberán et al., 2001); whereas, up to now it is the first time that both isomers of abscisic acid have been detected in buckwheat honey.

3.2.2. Flavonoid contents

The same HPLC analysis showed in all samples a variable amounts of lipophilic flavonoid aglycones and their total content was 345.4 μg/100 g. Most of the identified flavonoids were typical constituents of propolis, in particular the flavanones pinobanksin and pinocembrin, the flavones apigenin, chrysin and galangin. These compounds are often the main flavonoids in honey and so they are not useful as botanical origin markers. Their content in honey depend on the degree of propolis contamination in the hive and beeswax (Tomás-Barberán et al., 2001). In the samples these propolis-derived compounds represented 65% of the total flavonoids, with an average content of 232.5 μg/100 g. As shown in Table 4, pinobanksin was the main flavonoid with an average of 80.5 μg/100 g and representing the 21% of the total flavonoid content.

The HPLC–UV chromatogram of the honey extracts revealed also the presence of other three flavonoids, quercetin (peak 13), kaempferol (peak 16) and isorhamnetin (peak 17). Generally these compounds are related to the nectar origin, but they have been previously found in many honeys from different geographical and botanical origin (Estevinho et al., 2008; Martos et al., 1997; Tomás-Barberán et al., 2001; Truchado et al., 2008) suggesting that their potential use as markers can be limited. The main nectar-derived flavonoid in all samples was quercetin, ranging from 8.5% to 28.2% of the total flavonoid content, whereas isorahmnetin, along with chrysin, was present in the smallest amounts (7.6%). Besides, same samples did not contain this flavonoid.

Table 4
Phenolic compounds and abscisic acid contents in the different buckwheat honeys analysed. Values are μg/100 g honey. Different letters in the same line indicate significantly different values ($p \leqslant 0.05$).

#	Compounds	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10
Phenolic acids											
1	Protocatechuic acid[1]	102.3^{b}	20.5^{e-f}	$n.d.^{g}$	14.1^{f}	23.4^{d-f}	18.6^{f-e}	27.8^{d-e}	35.7^{d}	32.6^{c}	127.0^{a}
2	*p*-Hydroxybenzoic acid[1]	92.4^{f}	888.1^{a}	139.5^{f}	$447.7^{c,d}$	$611.3^{b,c}$	$717.8^{a,b}$	542.5^{b-d}	708.6^{b}	$189.6^{d,e}$	$262.5^{e,f}$
3	*p*-Hydroxyfenilacetic acid[1]	221.4^{a}	59.6^{c}	$n.d.^{d}$	144.7^{b}	55.1^{c}	67.4^{c}	57.8^{c}	$36.8^{c,d}$	$24.6^{c,d}$	60.3^{c}
4	Caffeic acid[1]	53.0^{e}	$64.3^{d,e}$	$n.d.^{f}$	$64.0^{d,e}$	87.8^{c}	$75.5^{c,d}$	89.1^{c}	54.3^{e}	189.1^{a}	214.6^{b}
5	Unidentified compound[1]	60.3^{g}	58.3^{g}	385.9^{d}	524.3^{c}	320.7^{e}	818.0^{b}	311.5^{e}	1624.4^{a}	70.5^{f}	34.3^{g}
6	Syringic acid[1]	31.7^{b}	$n.d.^{c}$	$n.d.^{c}$	$n.d.^{c}$	$n.d.^{c}$	$n.d.^{c}$	$n.d.^{c}$	$n.d.^{c}$	$n.d.^{c}$	45.3^{a}
7	*p*-Coumaric acid[1]	322.4^{h}	1150.3^{b}	58.5^{i}	548.8^{f}	844.1^{c}	764.5^{d}	740.6^{e}	1297.1^{a}	562.5^{f}	378.0^{g}
8	Ferulic acid[1]	161.2^{c}	173.6^{c}	$n.d.^{g}$	60.2^{f}	114.2^{d}	$n.d.^{g}$	79.9^{e}	$n.d.^{g}$	206.9^{b}	249.5^{a}
9	Isoferulic acid[1]	$63.7^{b,c}$	$n.d.^{f}$	$n.d.^{f}$	$38.4^{d,e}$	35.6^{e}	$n.d.^{f}$	$53.6^{c,d}$	$n.d.^{f}$	97.2^{a}	78.5^{b}
10	Benzoic acid[1]	288.4^{a}	225.3^{b-d}	39.5^{f}	198.9^{c-e}	$188.7^{d,e}$	165.7^{e}	$231.4^{b,c}$	167.9^{e}	$257.7^{a,b}$	168.1^{e}
Abscisic acids											
11	*trans–trans* Abscisic acid[1]	$49.9^{b,c}$	265.2^{a}	$34.6^{b,c}$	216.5^{a}	$155.1^{a,b}$	235.1^{a}	227.7^{a}	250.7^{a}	$94.1^{b,c}$	$53.2^{b,c}$
12	*cis–trans* Abscisic acid[1]	172.0^{d}	284.6^{a-c}	18.6^{e}	339.3^{a}	201.1^{b-d}	$196.6^{c,d}$	381.4^{a}	$305.5^{a,b}$	172.9^{d}	131.7^{d}
Flavonoids											
13	Quercetin[2]	46.9^{b-d}	$67.8^{a,b}$	8.8^{e}	$62.6^{b,c}$	39.6^{d}	$64.5^{b,c}$	53.3^{b-d}	88.3^{a}	53.4^{b-d}	$44.6^{c,d}$
14	Apigenin[2]	$20.5^{c,d}$	25.8^{b-d}	$13.8^{c,d}$	55.2^{a}	$41.4^{a,b}$	$22.2^{c,d}$	56.5^{a}	$38.9^{a,b}$	31.5^{b-d}	$33.8^{b,c}$
15	Pinobanksin[3]	$55.5^{d,e}$	$51.2^{d,e}$	10.8^{f}	$54.4^{d,e}$	59.1^{d}	44.4^{e}	106.8^{c}	21.1^{f}	250.1^{a}	151.4^{b}
16	Kaempferol[2]	$18.4^{d,e}$	21.9^{c-e}	14.5^{e}	$46.7^{a,b}$	$19.3^{d,e}$	$41.4^{b,c}$	63.2^{a}	37.6^{b-d}	$41.1^{b,c}$	27.2^{b-e}
17	Isorhamnetin[2]	20.6^{a-c}	$15.8^{b,c}$	$n.d.^{c}$	39.1^{a}	$21.2^{a,b}$	$23.4^{a,b}$	40.9^{a}	$n.d.^{c}$	$24.4^{a,b}$	$29.3^{a,b}$
18	Chrysin[2]	$40.6^{d,e}$	27.7^{e-g}	19.2^{g}	44.0^{d}	35.7^{d-f}	$24.1^{f,g}$	75.2^{c}	$22.4^{f,g}$	128.2^{b}	90.8^{a}
19	Pinocembrin[3]	$23.7^{d,e}$	$18.5^{e,f}$	11.1^{f}	31.1^{d}	32.4^{d}	$17.7^{e,f}$	60.6^{c}	12.9^{f}	117.6^{a}	98.8^{b}
20	Galangin[2]	17.4^{c}	12.3^{c}	10.9^{c}	21.1^{c}	18.9^{c}	11.6^{c}	36.6^{b}	14.1^{c}	54.6^{a}	50.6^{a}
	Total phenolic contents	1786.1^{g}	3363.8^{b}	754.9^{g}	$2857.4^{d,e}$	2824.1^{e}	$3240.7^{b,c}$	3088.7^{b-d}	4695.1^{a}	2324.1^{c-e}	2148.7^{f}

n.d., not detected.
[1] Calculated using the calibration curve of ferulic acid at $\lambda = 280$ nm.
[2] Calculated using the calibration curve of chrysin at $\lambda = 330$ nm.
[3] Calculated using the calibration curve of chrysin at $\lambda = 280$ nm.

Table 5
Volatile compounds identified in investigated honey samples, expressed as percent of total chromatogram area.

Peak	Compounds	RI	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10
1	Dimethyl sulfide	<800	0.33	0.12	0.04	0.20	0.18	–	0.67	0.04	0.55	2.95
2	Octane	<800	2.41	0.49	0.24	0.27	1.18	0.13	0.40	0.07	1.39	1.37
3	Acetone	<800	1.23	–	–	–	–	–	–	–	0.41	0.75
4	1-Octene	<800	0.49	–	–	–	–	–	–	–	0.28	–
5	Butanal	<800	–	0.36	0.48	0.24	0.35	0.41	0.26	0.24	0.28	–
6	2-Propanol, 2-methyl	804	1.04	–	–	0.10	–	–	–		–	–
7	Butanal, 2-methyl–	809	0.33	2.08	3.15	2.21	4.70	3.54	2.14	3.20	2.37	0.89
8	Butanal, 3-methyl–	811	0.25	2.69	3.92	2.20	6.16	4.16	2.36	2.73	3.09	1.36
9	Ethanol	819	–	–	0.23	0.43	0.26	0.53	0.37	–	0.13	0.18
10	Furan, 2,5-dimethyl–	829	–	–	–	–	–	–	–	–	–	0.09
11	Pentanal	840	0.37	0.55	0.83	0.52	0.62	0.86	0.51	0.56	0.28	0.33
12	Amylene Hydrate	860	0.53	–	0.11	0.07	–	0.14	–	–	–	–
13	Trichloromethane	865	0.34	0.20	0.48	0.35	0.43	–	0.68	–	0.95	0.85
14	Toluene	872	–	0.40	0.08	0.05	0.28	0.10	0.15	0.17	0.22	0.32
15	Butanoic acid, 2-methyl–, ethyl ester	883	–	–	0.07	0.17	0.03	0.16	0.10	0.06	–	–
16	Disulfide, dimethyl	890	0.78	0.29	0.13	0.08	0.77	0.11	0.31	–	0.12	0.16
17	Butanoic acid, 3-methyl–, ethyl ester	892	–	–	0.20	0.42	0.08	0.49	0.28	0.13	–	–
18	Hexanal	898	0.36	0.11	0.09	0.07	0.23	0.05	0.10	0.04	0.13	0.08
19	2-Butenal, 2-methyl–	906	–	0.11	0.08	0.07	0.52	–	0.15	0.07	0.46	0.22
20	1-Propanol, 2-methyl–	920	0.27	–	0.19	0.22	0.04	0.25	0.04	0.03	0.05	0.18
21	Propanoic acid, 2-methyl	951	–	0.12	0.09	0.05	0.42	–	0.07	0.04	–	–
22	1-Butanol	986	–	0.16	–	–	0.06	–	0.12	0.02	–	0.12
23	2-Heptanone	1029	–	0.04	–	–	0.07	–	0.04	0.04	–	0.04
24	Heptanal	1033	0.42	0.11	0.11	0.06	0.21	0.08	0.12	0.03	0.18	0.08
25	2-Butenal, 3-methyl–	1042	0.94	–	0.07	0.06	0.14	–	0.08	–	0.16	0.16
26	D-Limonene	1045	–	0.04	0.09	0.04	0.03	0.05	0.02	0.10	0.11	0.21
27	1-Butanol, 2-methyl–	1072	–	0.69	1.07	1.72	1.31	1.31	1.08	0.82	1.28	4.39
28	Not identified	1074	1.58	0.72	–	1.15	0.19	–	1.38	–	1.69	–
29	3-Buten-1-ol, 3-methyl–	1121	0.75	–	0.14	0.09	0.15	0.20	0.06	–	0.53	0.36
30	1-Butanol, 3-methyl	1128	–	0.06	0.04	0.05	0.06	0.04	0.08	0.08	0.06	0.33
31	*o*-Cymene	1139	0.35	0.34	0.04	0.51	0.14	0.08	0.44	0.10	–	–
32	Octanal	1168	1.90	0.49	0.55	0.28	0.62	0.30	0.53	0.16	0.80	0.40
33	Butanoic acid, 3-methyl, 2 methylbutylester	1186	–	–	–	–	–	–	0.06	0.36	–	–
34	1-Pentanol, 2-methyl–	1191	0.17	-	0.04	0.03	–	0.04	–	–	–	–
35	2-Buten-1-ol, 2-methyl–	1207	–	–	–	–	0.12	-	0.1	-	0.39	0.42
36	2-Heptanol	1212	0.33	–	0.04	0.03	0.21	0.07	0.12	0.08	0.10	0.11
37	5-Hepten-2-one, 6-methyl	1218	0.11	0.08	0.06	0.05	0.09	0.10	0.06	0.04	0.21	0.07
38	1-Hexanol	1237	0.21	0.32	0.02	0.03	0.16	–	0.08	–	0.10	0.19
39	Dimethyl trisulfide	1242	0.12	0.19	0.04	0.01	0.12	0.04	0.06	0.02	0.05	0.05
40	3-Hexen-1-ol (Z)	1260	–	–	–	–	0.08	–	–	–	0.12	0.15
41	2-Nonanone	1262	0.22	0.22	0.08	0.05	0.23	0.16	0.15	0.24	0.22	0.33
42	Nonanal	1267	8.00	2.75	2.25	1.58	4.41	1.60	2.69	0.86	5.46	1.59
43	Ethanol, 2-butoxy	1271	–	–	0.04	0.03	–	–	0.04	–	0.22	–
44	Benzene, (2-methyl-1-propenyl)–	1296	2.44	2.40	0.48	5.36	0.42	0.15	4.67	0.11	1.13	–
45	*cis*-Linalool oxide	1302	1.59	4.50	2.92	7.46	1.71	1.90	5.29	2.79	1.22	2.82
46	Acetic acid	1306	1.28	0.35	0.49	0.31	0.76	0.31	0.45	0.19	2.08	2.43
47	*trans*-Linalool oxide	1324	1.12	2.04	1.24	3.32	0.88	1.29	2.29	1.25	1.04	1.75
48	5-Hepten-2-ol, 6-methyl	1326	0.27	–	–	–	–	–	–	–	0.21	0.56
49	Ethanone, 1-(2-furanyl)	1345	3.07	1.06	3.28	2.22	1.92	3.26	1.18	5.31	2.44	0.77
50	Decanal	1348	3.98	1.16	1.44	0.70	1.39	0.68	1.16	0.64	1.42	0.76
51	Benzaldehyde	1354	15.35	6.64	10.23	5.31	11.63	5.19	5.44	1.26	4.42	2.72
52	2-Bomene	1357	–	1.15	–	–	–	0.86	–	0.56	–	–
53	3-Heptanol	1365	0.40	–	0.24	0.12	–	0.25	–	–	–	–
54	2-Nonanol	1369	0.15	–	0.16	0.18	0.11	0.09	–	0.09	–	0.23
55	Lilac aldehyde (isomer I)	1373	0.42	0.35	0.28	0.49	0.92	0.46	0.37	0.18	–	–
56	Lilac aldehyde (isomer II)	1382	–	0.50	0.32	0.61	1.12	0.46	0.53	0.21	–	0.12
57	Linalool	1388	1.78	0.33	0.21	0.64	1.07	0.31	0.73	0.18	7.90	27.63
58	Lilac aldehyde (isomer III)	1388	–	0.24	0.14	0.27	0.52	0.21	0.17	0.11	–	–
59	Furfural, 5-methyl	1392	0.70	–	1.32	0.66	0.24	0.85	0.2	1.43	0.25	0.25
60	1-Octanol	1393	1.71	0.49	0.52	0.40	0.51	0.39	0.45	0.36	0.78	1.26
61	2(3H)-Furanone, dihydro-3-methyl	1396	–	0.44	0.42	0.35	0.35	0.49	0.32	0.51	0.58	–
62	Isophorone	1398	–	0.23	0.13	0.55	0.14	–	0.98	–	–	0.24
63	Lilac aldehyde (isomer IV)	1404	0.40	0.47	0.30	0.50	1.05	0.40	0.45	0.22	–	–
64	2(3H)-Furanone, dihydro-5-methyl	1412	–	0.28	0.15	0.09	0.08	0.12	0.12	0.16	0.12	0.18
65	2H-pyran-2-one, tetrahydro	1415	–	3.70	2.55	1.37	2.97	2.76	1.73	2.86	2.37	0.64
66	*p*-Menth-1-en-4-ol	1421	0.24	0.04	0.02	0.15	0.16	0.02	0.17	0.05	0.06	0.14
67	3-Cyclohexene-1-acetaldehyde, α,4-dimethyl–	1327	–	3.82	1.00	1.24	1.52	1.03	1.74	1.57	–	–
68	Hotrienol	1437	1.99	14.13	2.53	6.41	4.48	2.27	8.97	1.15	3.18	4.56
69	Butanoic acid	1443	0.29	1.34	2.06	1.46	0.72	1.77	1.53	2.82	0.89	0.59
70	Phenylacetaldehyde	1448	6.70	6.88	7.61	3.79	3.87	8.42	3.30	0.89	2.98	2.08
71	Acetophenone	1453	–	–	–	–	–	–	–	–	–	0.34
72	Furfuryl alcohol	1474	0.98	0.56	0.66	1.24	0.91	0.70	1.35	0.98	1.41	2.56
73	Butanoic acid, 3-methyl	1480	3.85	11.38	22.62	14.70	14.07	22.29	13.79	38.49	11.03	5.92
74	4-Oxoisophorone	1489	0.59	1.42	0.48	0.51	0.86	0.62	0.76	1.06	0.31	0.24

Table 5 (*continued*)

Peak	Compounds	RI	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10
75	Verbenol	1493	0.32	0.19	–	0.29	0	–	0.25	–	0.23	–
76	α-Terpineol	1503	2.01	0.11	0.17	0.55	0.84	0.14	0.78	0.15	0.82	1.33
77	Borneol	1504	1.08	0.54	0.21	0.80	0.6	0.20	0.62	0.22	–	–
78	Nephthalene	1523	–	–	0.27	0.11	–	1.40	–	–	–	–
79	2(5H)-Furanone	1532	–	–	0.14	0.08	0.07	0.25	0.05	0.34	–	–
80	Pentanoic acid	1535	0.09	1.57	5.24	3.33	1.20	4.92	2.88	7.70	0.57	0.53
81	Linalool oxide III	1538	0.34	0.19	–	0.49	–	–	0.39	–	0.22	0.57
82	Ethanone, 1-(3-methylphenyl)–	1553	0.43	0.23	0.10	0.12	–	–	0.11	–	–	–
83	Benzoic acid, 2-hydroxy–, methyl ester	1556	1.10	0.24	0.11	0.36	0.06	0.08	0.08	0.05	4.01	4.12
84	1-Decanol	1565	0.94	0.56	0.16	1.39	0.42	0.16	1.20	0.11	0.54	0.50
85	Pentanoic acid, 3-methyl	1579	–	–	0.17	–	0.65	–	–	–	–	–
86	Pentanoic acid, 4-methyl	1588	–	–	0.11	0.11	0.07	0.15	–	0.25	–	–
87	Benzyl alcohol, α-methyl	1594	–	–	–	–	0	–	–	–	–	0.28
88	1-Octanone, 1-(2-furanyl)–	1594	–	–	–	0.09	0	0.17	–	0.82	-	–
89	β-Damascione	1446	0.28	0.21	–	0.39	0.34	–	0.36	–	–	–
90	3,6-Octadien-1-ol, 3,7-dimethyl, (Z)	1601	–	–	–	–	–	–	–	–	0.55	–
91	Nephthalene, 1-methyl (isomer I)	1612	–	–	0.13	–	–	0.40	–	–	–	–
92	Hexanoic acid	1625	0.44	0.24	0.26	0.33	0.48	0.19	0.27	0.17	0.77	0.53
93	*p*-Cymen-8-ol	1627	2.97	3.74	0.45	6.92	0.39	0.21	4.52	0.08	1.66	0.10
94	5,9-Undecadien-2-one, 6,10-dimethyl (E)	1637	–	0.02	0.06	–	0.05	0.07	0	–	0.22	–
95	Benzylalchol	1646	1.99	2.22	0.36	0.27	0.91	0.12	1.04	0.21	3.95	3.54
96	Phenylethyl alcohol	1675	2.69	0.72	1.03	1.17	1.71	3.61	1.59	0.34	4.11	2.45
97	Benzyl nitrile	1684	0.32	0.36	0.14	0.10	4.63	–	0.4	–	0.17	0.10
98	Methyl 2-furoate	1744	0.20	0.56	1.21	0.62	0.53	1.30	0.54	2.39	0.16	–
99	Phenol	1756	0.26	0.19	0.32	0.08	0.10	0.26	0.05	0.12	0.27	0.20
100	Benzaldehyde, 4-methoxy–	1761	0.43	0.48	0.93	0.69	0.34	2.36	0.48	2.23	0.64	0.72
101	2-Propenal, 3-phenyl	1773	0.25	0.16	0.17	0.15	0.35	0.26	0.17	–	0.91	0.52
102	Octanoic Acid	1809	2.14	0.51	0.62	0.89	0.86	0.60	1.13	0.15	1.96	1.54
103	5-Methyl-2phenyl-2hexanal	1813	–	–	0.25	0.28	0.09	0.94	0.21	0.41	–	–
104	*p*-Cresol	1821	0.99	2.52	4.72	2.76	2.32	5.70	2.26	6.38	1.48	0.77
105	2(3H)-Furanone, 5-heptydihydro	1866	–	–	–	–	–	–	–	–	0.08	0.24
106	Eugenol	1895	–	–	–	–	0.63	–	–	–	–	–
107	Nonanoic acid	1904	3.02	1.25	0.56	0.60	1.36	–	1.00	–	3.30	1.94
108	Tymol	1920	–	–	–	0.13	–	–	1.86	–	2.40	0.21
109	Ethanone, 1-(2-aminophenyl)–	1929	0.26	1.46	1.15	0.77	1.30	2.22	1.05	1.16	0.66	0.88
110	Carvacrol	1941	0.74	0.53	–	0.89	–	–	0.65	–	–	–
111	3,5-Dimethoxybenzaldehyde	1984	–	0.31	0.49	0.18	0.20	0.44	0.18	0.24	–	–
112	2-Propen-1-ol, 3-phenyl	1990	–	–	–	–	–	–	–	–	0.90	0.50
113	Decanoic acid	1995	0.40	-	-	0.15	0.16	–	0.31	–	0.43	0.33
114	Phenol, 3,4,5-trimethyl	2043	1.58	0.51	1.17	0.36	–	0.72	-	–	–	–
115	Benzofuran, 2,3.dihydro–	2086	0.21	0.40	0.13	0.07	0.16	0.16	0.17	0.13	0.46	0.17
116	Benzoic acid	2120	0.64	0.28	0.15	0.46	–	–	1.25	–	–	–
117	5-Hydroxymethylfurfurale	2171	–	–	–	–	–	–	–	0.32	–	–
118	Isobutyl phtalate	2221	0.77	0.08	0.15	0.09	0.15	0.43	0.11	0.23	0.28	0.39

Phenolic analyses of buckwheat honey were of particular interest because of the well-studied antioxidant activity of the corresponding plant source. The antioxidant proprieties of buckwheat (*F. esculentum*) have been attributed to high levels of a specific flavonoid, rutin. However the phenolic profile differs throughout a plant; thus the phenolic pattern of the plant nectar, and the corresponding honey, might be quite different, from that of other plant tissues (Kalinová, Triska, & Vrchotov, 2006). Therefore, rutin was not found in our buckwheat honeys, according to other authors (Gheldof et al., 2002).

In this work only flavonoid aglycones were identified, supporting the suggestion that only these derivate were present in honey because the nectar flavonoid glycosides were fully hydrolyzed by the hydrolytic enzymes present in bee secretions (Gil, Ferreres, Ortiz, Subra, & Tomás-Barberán, 1995).

3.3. Determination of volatile compounds in buckwheat honey

Table 5 lists the volatile compounds identified by their GC retention and their mass spectra data in the investigated honey samples. More than 100 volatile compounds were positively identified and most of them were present in all honey samples even if in different amount.

Among the volatile components detected in all samples, several furanic compounds such as furfural, 1-(2-furanyl)-ethanone, 5-methylfurfural, 5-hydroxymethylfurfural (HMF) and furfuryl alcohol were also present. Relative TIC areas for these compounds were quite different, even though furfural was the most abundant in all honeys with an average up to 35%, excepting in the B-10 sample (11.09%) (data not reported). Furanic compounds are commonly produced either by heating or storage (Visser, Allen, & Shaw, 1988; Wootton, Edwards, & Faraji-Haremi, 1978). Also the mild heating undergone by samples during SPME sampling, which is recommended in order to improve the extraction yield and to reduce the equilibrium time, could be partially responsible for some of them. For these reasons, furfural was not taken into account for the final percent quantification of the honey volatile composition. In this way it was possible to highlight also the presence of other minor compounds in honey samples, as showed in Table 5.

Some compounds present in buckwheat honeys, such as ethanol, toluene, *trans*-linalool oxide, hotrienol, benzeneacetaldehyde have been already reported as common components of various honeys (Alissandrakis et al., 2007; Baroni et al., 2006; Soria et al., 2003). 3-Methylbutanoic acid was the major compound in most of the samples, with a percentage ranging from 3.85% (B-1) to 38.51 (B-8)%. This result is in agreement with other studies on buckwheat honey, where 3-methylbutanoic acid showed the

highest concentration (Plutowska, Chmiel, Dymerski, & Wardencki, 2011; Wolski, Krzysztof, Rybak-Chmielewska, & Kedzia, 2006) and was also included among the predominant odorants that contribute to the typical malty flavour of buckwheat honey (Zhou, Wintersteen, & Cadwallader, 2002). The other compounds present in high amounts, were found in different percentages in all the studied samples. In particular, B-10 was characterised by a high content of linalool (27.63%) followed by 3-methylbutanoic (5.92%), hotrienol (4.56%), 2-methyl-1-butanol (4.39%), 2-hydroxy-benzoic acid, methyl ester (4.12%), and benzylalcohol (3.54%). Also B-9 showed the same highest compounds, even though 3-methylbutanoic acid was the major component (11.05%). Sample B-8 showed a different profile where the highest compounds were pentanoic acid (7.70%), *p*-cresol (6.38%) and 1-(2-furanyl)-ethanone (5.32%). As reported by Zhou et al. (2002), *p*-cresol is another important aroma compound that contribute to the overall malty flavour of buckwheat honey. This sample, followed by B-6 (5.72%), was the only one with a high percentage of this compound and it was also the honey with the highest amount of the 3-methylbutanoic acid (38.51%). On the other hand, B-8 showed a high number of furan derivatives in quite high percentage, such as 1-(2-furanyl)-ethanone, 2-furanmethanol, 5-methylfurfural, dihydro-3-methyl-2(3H)-furanone, dihydro-5-methyl-2(3H)-furanone, fufuryl alcohol, 2(5H)-furanone, 1-(2-furanyl)-1-octanone and 5-hydroxymethylfurfurale. This last compound was found only in the B-8 sample and usually it is used as a marker of excessive heating. Among the other samples, B-3 and B-6 showed a similar profile with a high 3-methylbutanoic acid content ranging from 22.36% and 22.66%, followed by phenylacetaldehyde, benzaldehyde, penatnoic acid and *p*-cresol. B-4, B-5 and B-7 had a similar volatile profile with a medium content of 3-methylbutanoic acid, ranging from 13.79% to 14.70% and a high content of hotrienol, from 4.48% to 8.97%, compared with the other samples. Despite of other honeys, B-1 and B-2 showed a different percentage distribution of the volatile compounds. In particular, B-1 had benzaldehyde as principal component (15.35%) and a low content of 3-methylbutanoic acid (3.85%). Benzaldehyde, along with other benzoic derivatives, has been reported as ubiquitous in most honeys of a wide range of floral sources. B-2 showed a high amount of hotrienol (14.14%) followed by 3-methylbutanoic acid (11.39%) and phenylacetaldehyde (6.88%).

In all samples were found 3-methylbutanal and 2-methylbutanal, ranging from 0.25% to 6.16% and from 0.33% to 4.70%, respectively. These compounds have malty and pungent notes and play an important role in the characteristic odour of buckwheat honey (Plutowska et al., 2011), with the 3-methylbutanal the most important one. 2- and 3-Methylbutanal, commonly found in barley malt (Fickert & Schieberle, 1998), are known to be Strecker aldehydes and their presence in honey is associated with the Maillard browning reactions (Overton & Manura, 1994; Parliament, 1989). As the processing of honey involved several heating steps, the high amounts of compounds 2- and 3-methylbutanal presented in buckwheat honeys compared with some other honeys (Plutowska et al., 2011) suggested that buckwheat honey might contain a higher abundance of Strecker degradation precursors, such as amino acids, which would result in a honey with an aroma resembling that which develops upon heat promoted chemical reactions that occur during the malting of barley. The presence of other Maillard reaction products such as phenylacetaldehyde and dimethyl trisulfide in our samples supports this hypothesis. Among samples, B-1 showed the lowest content of 2- and 3-methylbutanal.

Also acetic acid, that has been previously proposed as being indicative of honeydew by Campos, Nappi, Raslan, and** Augusti (2000), was present in all samples even though in higher amount in B-1, B-9 and B-10 honeys. Besides, only in these three samples was not found the 3,5-dimethoxybenzaldehyde, another compound suggested to be present in buckwheat honey by Wolski et al. (2006).

4. Conclusion

Since an exaustive characterisation of the buckwheat honey is not yet available, this study proposes a preliminary but comprehensive and detailed evaluation of composition and properties of that botanic origin. Besides the traditional descriptive analyses as pollen spectra and physicochemical proprieties, the aromatic and antioxidant profile were also included in order to improve the monofloral assessment of buckwheat honey.

Indeed volatile compounds, because of their relation to aroma and hence to sensory acceptance of honey by the consumers, play an important role in honey authentication. On the other hand, the assessment of phenolics and other components in honey that may be responsible for its antioxidant effects have to be considered in order to improve our knowledge about honey as nutraceutical source.

First of all, the analytical results obtained for our honeys reveal the presence of three poorly pure samples (B-1, B-9 and B-10) which despite of their label cannot be useful for the characterisation of the buckwheat, presenting a strong blend with honeydew honey. The volatile analysis of the remaining samples showed more than 100 volatile compounds with the 3-methylbutanoic acid as principal one. This molecule in combination with 2- and 3-methylbutanal and pheynalcetaldehyde contribute to the typical buckwheat aroma of our samples. In the same honey samples, the phenolic profile was similar and showed *p*-hydroxybenzoic and *p*-coumaric acids as the main components. On these bases, the phenolic pattern and a significant content of 3-methylbutanoic acid in the aromatic profile could be treated as possible fingerprints of buckwheat honey, in the course of a preliminary estimation of the quality of this unifloral honey.

In conclusion, our approach, using all the parameters previously mentioned, provide an entrance to further research with a larger number of samples in order to improve the interest and knowledge about this honey and its quality profiles.

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